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An Atypical Case of Fanconi Anemia in Elderly Sibs

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We describe a 56-year-old woman suspected of Fanconi anemia on the basis of the following clinical findings: microcephaly, short stature, congenital deafness, and the clinical findings in her deceased brother. Hematologic or other signs of malignancy were absent. The diagnosis was confirmed by demonstrating hypersensitivity of her lymphocytes to mitomycin C (MMC). Cell fusion experiments indicated that the patient belongs to complementation group A. The patient's brother died at the age of 50 of heart and renal failure, and anemia. He had clinical findings similar to those of his sister, and a horseshoe kidney. From 31 years on he had thrombocytopenia and leucopenia. Both patients had insulin-dependent diabetes mellitus. A chromosomal breakage test carried out elsewhere before his death failed to demonstrate MMC hypersensitivity of his lymphocytes, which led to the investigation of his sister. To our knowledge these two cases are the oldest Fanconi anemia patients reported thus far. Am. J. Med. Genet. 68:362–366, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: Fanconi anemia; inherited aplastic anemia; genetic subtype; clinical variability

INTRODUCTION

Fanconi anemia (FA) is an autosomal recessive disease characterized by aplastic anemia, usually with onset at the age of 5–10 years and frequently associated with a variety of congenital anomalies. FA patients have chromosome instability and hypersensitivity to crosslinking agents such as mitomycin C(MMC) and diepoxybutane and an increased risk of malignancies [Schroeder et al., 1964; Sasaki and Tonomura, 1973].

FA is genetically heterogeneous: at least five complementation groups (separate disease genes) are distinguished based on the restoration of crosslinker tolerance in fusion hybrids from cell lines derived from unrelated FA patients [Strathdee et al., 1992a; Joenje et al., 1995]. The gene responsible for FA group C (*FAC*) has been cloned [Strathdee et al., 1992b], but the function of the FAC protein in the cell is still unknown.

The congenital anomalies in FA patients may vary in site and severity and some patients do not have any malformations at all, which makes early diagnosis difficult. Most patients without characteristic physical findings were not diagnosed until after the onset of hematologic abnormalities [Glanz and Fraser, 1982; Alter and Potter, 1983; Kwee and Kuyt, 1989; Giampetro et al., 1991; Young and Alter, 1994]. Clinical variation may be correlated with complementation group or type of mutation, but at present no such information is available. In general the prognosis is poor. Most reported patients died before the age of 20–30 years. We describe in this report a 56-year-old woman with FA who could be assigned to complementation group A based on cell fusion experiments. Her elder brother, who died at the age of 50, had more clinical signs and presumably also had FA, even though the diagnosis FA could not be confirmed cytogenetically before his death. To our knowledge these two cases are the oldest FA patients documented in the literature thus far [Young and Alter, 1994].

CLINICAL REPORTS

Patient 1

The proposita was born in 1938 after an uneventful pregnancy of 7 months. Her birthweight was 750 g. At the age of 3 years she had one epileptic episode. She had frequent respiratory and middle-ear infections. Her mother described her as a hyperkinetic child. Since the age of 9 years she has stayed in several institutions. Her detailed clinical history is not known. At 45 years she had the following signs: microcephaly (head circumference 42 cm), café-au-lait spots, short stature (1.40 m), deaf-mutism, and mental retardation. Facial traits included hypotelorism, upward slant of the eyes, midface hypoplasia, bulbous nose (Fig. 1). Also noted

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Fig. 1. Frontal and lateral view of patient 1.

was clinodactyly of the 5th fingers. She has insulin-dependent diabetes mellitus and apparently hallucinations that are treated with neuroleptics. HbF was 4.3%. She did not have any other haematological findings of FA and there were no signs of malignancies. She had no growth hormone deficiency. X-ray studies of the hands showed a fusion of the os lunatum and os triquetrum, clinodactyly of the 5th fingers, and slight brachydactyly of all fingers.

Because there was a similar case in the family with, in addition, hematological findings (see below) the diagnosis of FA was suspected and subsequently confirmed with laboratory investigations.

Patient 2

He was the elder brother of patient 1 and was born in 1935 after an uneventful pregnancy of 7 months. The delivery was difficult due to severe oligohydramnios. Birthweight was about 1,000 g. In addition to the findings described for his sister he had a horseshoe kidney. From the age of 10 years he stayed at different institutions for the deaf and later in institutions for the mentally retarded. Since the age of 31 years he has had thrombocytopenia and leucopenia. At 33 years he had a streptococcal infection and haematuria with temporary uraemia. He also developed insulin-dependent diabetes mellitus. He was treated with neuroleptics because of apparent hallucinations. Since the age of 45 he had hypertension and a systolic heart murmur. Examination at 48 years showed microcephaly (head circumference 44 cm), small stature (height 1.50 m), ptosis, slight thoracic kyphosis, several café-au-lait spots, and hypogonadism (Fig. 2). He developed the nephrotic syndrome, anemia, and leucopenia. FA was suspected at that age and cytogenetic investigation was done elsewhere but did not show hypersensitivity to MMC, so that the diagnosis was unconfirmed. In the next 2 years his diabetes became more difficult to manage, anemia progressed, and he developed renal failure. He died at 50 years because of heart failure. Autopsy was not performed. Further investigation was then continued on his sister (patient 1).

Family History

The patients are the first and second child, respectively, of healthy and nonconsanguineous Dutch parents. Three sibs of the patients are normal. The family history is unremarkable; only maturity onset diabetes mellitus is present in the family (two aunts of the patients).

LABORATORY INVESTIGATIONS

Cytogenetics

Chromosome aberrations were evaluated from coded Giemsa-stained metaphase preparations, prepared

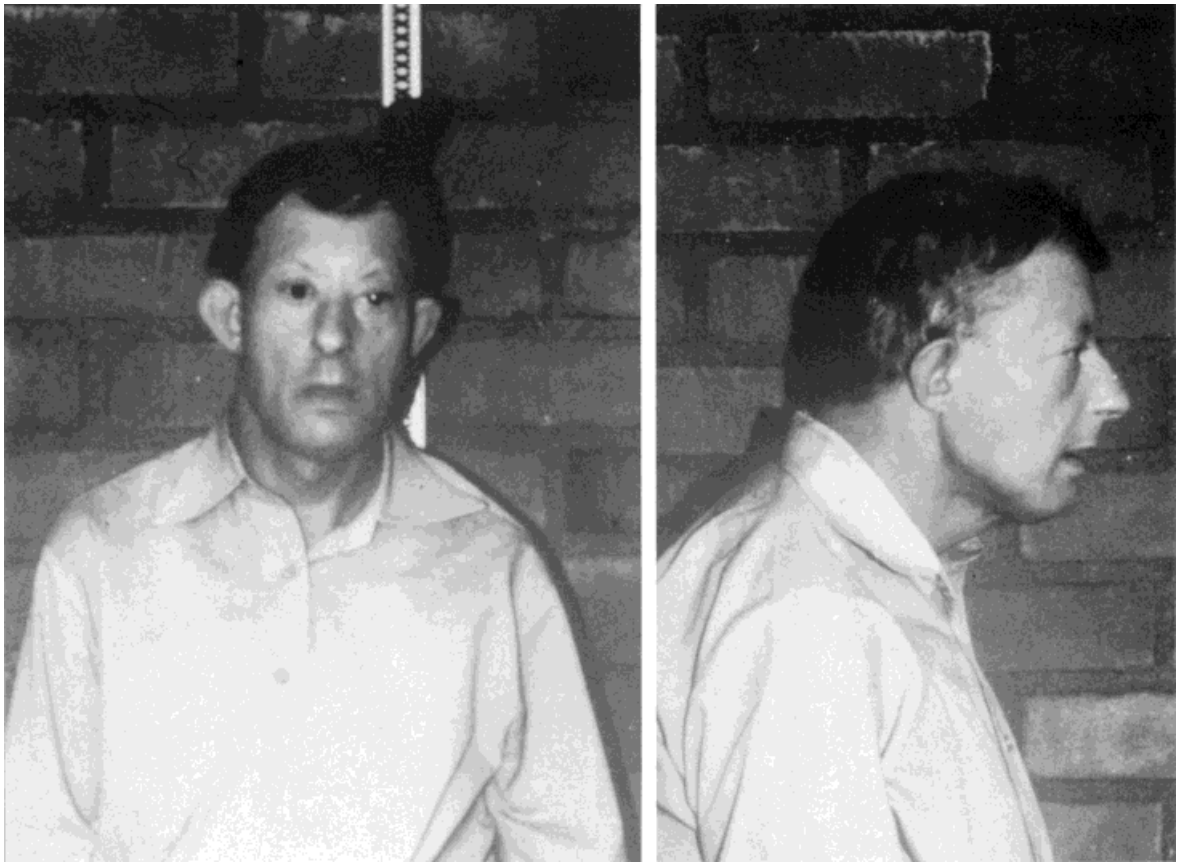

Fig. 2. Frontal and lateral view of patient 2.

from 72 hour phytohaemagglutinin-stimulated whole blood cultures of patient 1, as described before [Kwee et al., 1983]. Crosslinker sensitivity was assessed by including MMC at 0, 0.015, 0.045, and 0.1 μg/ml, in parallel with blood cultures from a 66-year-old healthy male control.

Lymphocyte Immortalization

Supernatant from the Epstein-Barr virus (EBV) producer line B-95/8 was used to establish immortalized B lymphocytes from patient 1. Sensitivity to MMC was assessed by determining drug-dependent growth inhibition over a 5-day period [Ishida and Buchwald, 1982].

Complementation Analysis

A fibroblast cell line was obtained from a skin biopsy of patient 1. VH-4 "TOR" (thioguanine and ouabain resistant) is a MMC-sensitive Chinese hamster cell line, previously shown to be genetically homologous to FA complementation group A [Arwert et al., 1991]. Cell fusion between fibroblasts of patient 1 and VH-4 TOR was performed as described earlier [Arwert et al., 1991]. A mixed population of hybrid clones (>100) was collected and used for MMC survival assays and for the determination of the chromosome content. Cytogenetic analysis and survival assays of hybrid cells were performed as described [Arwert et al., 1991].

RESULTS

On standard cytogenetic analysis the karyotypes of both patients were normal. Sensitivity to MMC, as determined in phytohaemagglutinin-stimulated blood cultures from patient 1 and a healthy control are summarized in Figure 3. The results show that the patient's lymphocytes were about 10-fold more sensitive to the clastogenic action of MMC than control lymphocytes, a degree of hypersensitivity that is similar to that of FA patients diagnosed previously in our laboratory. These results confirmed the clinically suspected diagnosis of FA in patient 1.

An immortalized EBV-transformed lymphoblast cell line established from the patient was tested for MMC sensitivity by a growth inhibition test. As shown in Figure 4, the sensitivity of this cell line was similar to that observed in two other control lines, i.e., about 10- to 20-fold less than three cell lines established from other FA patients.

Owing to its resistance to MMC, this cell line could not be used to determine the patient's complementation group by the procedure used by Joenje et al. [1995]. However, the MMC-sensitive Chinese hamster cell mutant VH-4 TOR can be used to determine whether an unclassified fibroblast strain belongs to the FA complementation group A or not [Arwert et al., 1991]. Figure 5

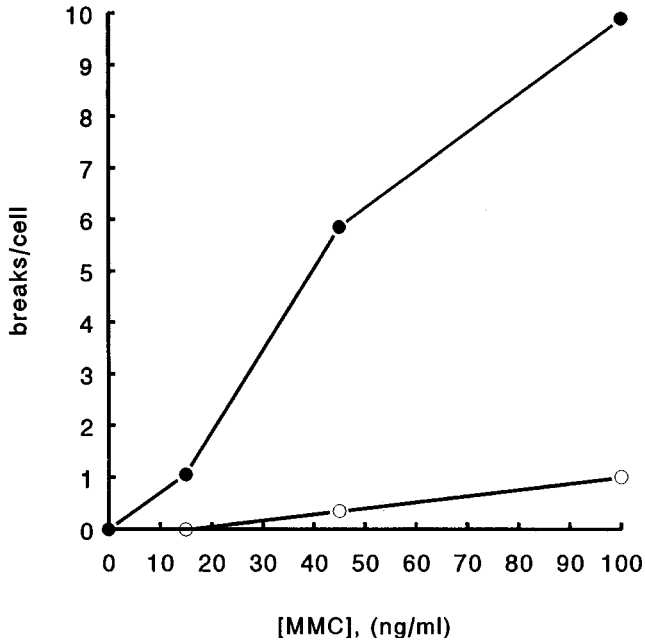

Fig. 3. Chromosomal breakage in 72 h lymphocyte cultures from patient 1 (●) vs. a healthy control (○).

shows MMC sensitivity of a hybrid cell population obtained after fusion of VH-4 TOR with fibroblasts obtained from patient 1. Even though these hybrids were somewhat more resistant to MMC than the VH-4 TOR fusion parent, their level of MMC resistance was similar to that of hybrids generated with fibroblast known to belong to complementation group A (HSC402 and GM1309), and well below the level of MMC resistance

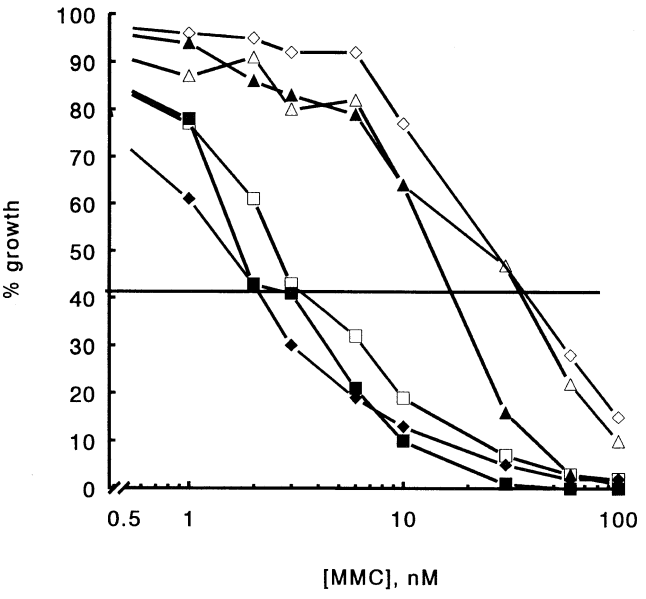

Fig. 4. MMC-induced growth inhibition in lymphoblasts. △ and ◇, lymphoblasts from two healthy individuals; □, FA complementation group A lymphoblasts (HSC72); ■ and ◆, FA patients from unidentified complementation group; ▲, lymphoblasts from patient 1.

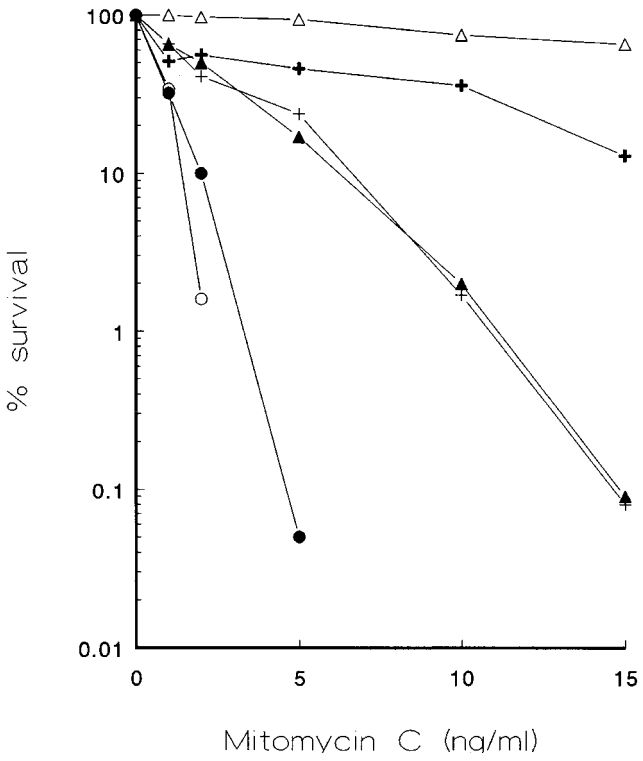

Fig. 5. Clonogenic survival of the following cell lines and fusion hybrids, as a function of treatment with MMC. △: V79, a normal Chinese hamster cell line; ○: VH-4 TOR, a MMC sensitive mutant of V79, homologous to FA-A; : fusion hybrid between VH-4 TOR and FA-A fibroblasts (GM1309); +: fusion hybrid between VH-4 TOR and normal control fibroblasts; ▲: fusion hybrid between VH-4 TOR and FA-A fibroblasts (HSC402). +: fusion hybrid between VH-4 TOR and fibroblasts from patient 1.

observed in hybrids generated with normal control fibroblasts. To exclude the possibility that loss of chromosomes harboring putative complementing genes were responsible for the observed lack of complementation, R-banded chromosome spreads from the hybrid cell population VH4 TOR x patient 1 fibroblasts was examined for the presence of human chromosomes. Since no specific chromosome loss was detected, lack of complementation could not be explained by specific chromosome loss. Therefore the data strongly suggest that patient 1 belongs to the FA complementation group A.

DISCUSSION

Our female patient has a few of the physical and none of the typical hematological findings of FA, except for a relatively high level of HbF. Her cells showed a high degree of hypersensitivity to MMC and the complementation test indicated that she belongs to complementation group A. However, hypersensitivity to MMC was not present in EBV-immortalized lymphoblasts from the patient. In our experience absence of hypersensitivity to crosslinkers in lymphoblast lines from otherwise sensitive FA patients is typically observed in 10–20% of patients diagnosed to have FA. Resistant cell lines presumably arise from resistant lymphocytes preexisting in certain patients exhibiting mosaicism [Kwee et al., 1983; Arwert and Kwee, 1989]; such resistant lymphocytes

may have a proliferative advantage during the immortalization procedure over cells that have the sensitive phenotype [Kwee et al., 1983]. To the best of our knowledge, loss of hypersensitivity to crosslinkers has not been observed in fibroblast cell lines obtained from skin biopsies of FA patients [Arwert and Kwee, 1989].

Lymphocytes of the proposita's brother failed to show MMC hypersensitivity in a single experiment with a single concentration of MMC; unfortunately the test could not be repeated. Because of similar symptoms as observed in his sister we assume in retrospect that he must have been affected also. As in his sister, mosaicism may have been present in his lymphoid cells so that only a minor part of his lymphocytes were MMC sensitive (typical FA cells), most of his cells reacting to MMC as normal cells.

Because of the rather atypical clinical findings in these patients the FA diagnosis was not suspected until the patients had reached an advanced age, even though their high age of survival by itself is unusual for FA and, to our knowledge, has not been reported before. Most FA patients die at a young age from severe pancytopenia, hemorrhage, sepsis, or a combination of these [Alter and Potter, 1983; Kwee and Kuyt, 1989; Young and Alter, 1994]. Acute leukemia has been the terminal event in about 5–20% of patients with FA [Alter and Potter, 1983]. Besides the hematological malignancies, there are other tumors described in FA patients, e.g., squamous cell carcinoma, adenocarcinoma of the stomach or colon, breast cancer, medulloblastoma, and Wilms tumor. The impression is that malignancies occur mainly in patients with late-onset marrow failure and longer survival [Kennedy and Hart, 1982; Alter and Potter, 1983].

Our patients had no overt findings of malignancy but were not examined internally so that the presence of an internal tumor cannot be excluded. Reported FA patients with a malignancy had a poor prognosis, as most of them died within 1 year after diagnosis.

The present report confirms that, because of the variability of physical and hematological symptoms in FA patients, the FA diagnosis can be readily missed and should be checked in any adult patients with a combination of the following findings: short stature, café-au-lait spots, microcephaly, congenital deafness, renal anomaly, and mental retardation, even in the absence of pancytopenia, leukemia, or malignancies that typically occur in FA patients at younger ages. Cytogenetic diagnostic methods (crosslinker-induced chromosomal breakage) are indicated in these situations.

Possible explanations for the mild expression and therefore better prognosis in terms of life expectancy for the present patients could be the presence of mosaicism, i.e., the presence of clastogen-resistant cells in addition to FA cells in the hematopoietic system [Kwee et al., 1983], or a specific kind of mutation associated with a mild FA phenotype. In addition, favourable genetic and/or environmental factors may have contributed to the relatively mild phenotype in these patients.

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